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**First report of a *bla*$_{NDM-5}$ harbouring *Escherichia coli* ST167 isolated from a wound infection in a dog in Switzerland**

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Running Title: F31:A4:B1 plasmid; NDM-5; *Escherichia coli* ST167; dog

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Sir,

The dissemination of carbapenemase producing Enterobacteriaceae (CPE) is a major concern for health care providers worldwide but so far, CPE are rarely described in companion animals [1].

The New Delhi metallo-β-lactamase (NDM-1) and its variants hydrolyse almost all clinically-available β-lactam antibiotics and thus represent a major challenge to the treatment of infections caused by bacteria carrying these resistance genes [2]. NDM-5 producing *E. coli* belonging to sequence type (ST) 167 or ST167 variants have been isolated previously from dogs in Algeria and Finland [3]. Here, we describe for the first time the isolation of a *bla*$_{\text{NDM-5}}$ harbouring *E. coli* isolated from a wound infection in a dog in Switzerland and present the complete genome sequence of this multidrug resistant isolate.

DNA extraction was performed using the GeneElute™ Bacterial Genomic DNA Kit (SigmaAdlrich, Buchs, Switzerland). Sequencing was done using two single-molecule real-time (SMRT) cells on a PacBio RS II (Pacific Biosciences, Menlo Park, CA, USA) and performed at the Functional Genomics Center Zurich (FGCZ), Switzerland. Sequencing reads of each cell were collected separately using the RS Filter Only protocol in the SMRT-portal (Pacific Biosciences) with 85 as polymerase read quality cut off and a minimal length of 50 bp. The collected reads of both cells were combined resulting in 279,307 reads with an average length of 7,389 bp and a maximum length of 61,011. In total, 2,063,810,221 bp were sequenced, corresponding to an approximate 400-fold coverage of the genome. The reads were assembled using the Canu assembler with the option "-pacbio-raw" and an estimated genome size of 5.0 Mbp. After polishing the first assembly by deleting overlapping regions in circular contigs, elimination of contigs present in larger contigs and eradication of phage DNA, the final assembly resulted in 6 circular contigs, one 4.8 Mbp chromosome and 5 contigs that were identified as plasmids by PlasmidFinder 1.3 (Table 1). The genome was annotated by the NCBI Prokaryotic Genomes Automatic Annotation Pipeline (GPAP) server. Multilocus sequence typing (MLST) was performed *in silico* using the Center for Epidemiology database (https://cge.cbs.dtu.dk/services/MLST E.coli#1 configuration). Phenotypic antibiotic resistance

was tested by the disk diffusion method or using the Etest (bioMérieux, Marcy l'Etoile, France), according to CLSI protocols and criteria (https://clsi.org).

*E. coli* 51008369SK1 was assigned by MLST to ST167.

Five plasmids belonging to the IncFII, IncI1, IncX4, IncI2 and IncFII-IncFIA-IncFIB replicon types, respectively, were detected (Table 1).The IncFII-IncFIA-IncFIB plasmid p51008369SK1_E was assigned by pMLST to the formula F31:A4:B1 and carried the $bla_{\text{NDM-5}}$ gene (Table 1).

By BlastN, 99% identity and 100% coverage was observed with plasmid pNDM-5-IT (GenBank accession no. MG649062), purified from *E. coli* ST167 Ec001 causing urinary tract infection in a patient in Italy [4]. The comparative analysis between p51008369SK1_E and pNDM-5-IT revealed that both plasmids harbour $bla_{\text{NDM-5}}$ in a complex integron containing an ISCR1 element and the *aadA2-dfrA12* resistance gene cassette. Both plasmids carry the toxin-antitoxin systems *sopAB*, *ccdAB* and *pemIK*, as well as the putative virulence factors *iucABCD*, *iutA*, and the arginine deaminase (ADI) cluster *arcABCD*, whose location on a plasmid has so far been unique to pNDM-5-IT [4]. Both plasmids contain a tunicamycin resistance gene *tmrB* in the same plasmid region. However, the *aac(3)'IIa-tmrB* segment which is present upstream of the *mph(A)-mrx-mph(R)* cluster in pNDM-5-IT is inverted and inserted downstream of *mph(R)* in p51008369SK1_E . As in pNDM-5-IT, the Tra locus for conjuagtion in p51008369SK1_E is only partially present and contains only *traX* and *finO* genes, which is consistent with failure to transfer the plasmid during conjugation experiments *in vitro* (data not shown).

*E. coli* 51008369SK1 was phenotypically resistant to ampicillin, amoxicillin-clavulanic acid, cefazolin, cefotaxime, cefepime and ertapenem. Furthermore, it was resistant to sulfamethoxazole-trimethoprim, azithromycin, gentamicin, chloramphenicol and tetracycline, consistent with acquired resistance determinants found on its five plasmids (Table 1). Nalidixic

acid and ciprofloxacin resistance corresponded to chromosomal point mutations found in *gyrA* that lead to amino-acid substitution in GyrA at position S83→L and D87→N (data not shown). F31:A4:B1 type plasmids are predominantly associated with the spread of $bla_{\text{CTX-M-15}}$ among *E. coli* isolates from humans and animals [5]. Our study provides evidence that this type of IncF plasmid may also promote the dissemination of $bla_{\text{NDM-5}}$. Furthermore, non-transmissible F31:A4:B1 type plasmids such as pNDM-5-IT and p51008369SK1_E not only confer selective advantages such as multidrug resistance and virulence to the host strain; the presence of addiction systems also ensures the stability and persistence of the plasmid within its specific host, *E. coli* ST167. The emergence of this successful epidemic clone harbouring $bla_{\text{NDM-5}}$ among veterinary *E. coli* isolates in Switzerland represents a public health threat that needs to be addressed not only in veterinary medicine, but due to the close contact of humans and companion animals, also in human healthcare settings.

**Nucleotide sequence**

Sequence and annotation data of the genome have been deposited at GenBank under accession numbers CP029973 for the chromosome and CP029974 to CP029978 for the plasmids.

**Declarations**

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**Competing Interests**: No conflicts of interest

**Ethical Approval:** Not required

**References**

1. Köck R, Daniels-Haardt I, Becker K, Mellmann A, Friedrich AW, Mevius D et al. Carbapenem-resistant Enterobacteriaceae in wildlife, food-producing, and companion animals: a systematic review. Clin Microbiol Infect 2018; 24; doi: 10.1016/j.cmi.2018.04.004.
2. Khan AU, Maryam L, Zarrilli R. Structure, genetics and worldwide spread of New Delhi Metallo-β-lactamase (NDM): a threat to public health. BMC Microbiol 2017; 17; 101. doi:10.1186/s12866-017-1012-8.
3. Grönthal T, Österblad M, Eklund M, Jalava J, Nykäsenoja S, Pekkanen K et al. Sharing more than friendship – transmission of NDM-5 ST167 and CTX-M-9 ST69 *Escherichia coli* between dogs and humans in a family, Finland, 2015. Euro Surveill 2018; 23; pii=1700497. 23, 27, pii=1700497.
4. Giufrè M, Errico G, Accogli M, Monaco M, Villa L, Distasi MA et al. Emergence of NDM-5-producing *Escherichia coli* sequence type 167 clone in Italy. Int J Antimicrob Agents 2018; 52:76-81. doi: 10.1016/j.ijantimicag.2018.02.020.
5. Li JJ, Spychala, CN, Hu F, Sheng JF, Doi Y. Complete nucleotide sequences of *bla*$_{\text{CTX-M}}$-harboring IncF plasmids from community-associated *Escherichia coli* strains in the United States. Antimicrob Agents Chemother 2015; 59: 3002-3007. DOI=10.1128/AAC.04772-14.

Figues and table

Table 1: Features of the five plasmids harboured by *Escherichia coli* 51008369SK1.

| Plasmid | Size | Replicon type | Antibiotic resistance determinants | Accession no. |
|---|---|---|---|---|
| p51008369SK1_A | 71,178 bp | IncFII | *bla*$_{CMY-2}$, *erm(B)* | CP029974 |
| p51008369SK1_B | 113,340 bp | IncI1 | *aadA1, bla*$_{TEM-30}$, *floR, sul1, sul2, dfrA1* | CP029975 |
| p51008369SK1_C | 33,826 bp | IncX4 | none | CP029976 |
| p51008369SK1_D | 61,284 bp | IncI2 | none | CP029977 |
| p51008369SK1_E | 99,465 bp | IncFII-IncFIA-IncFIB | *aac(3)-IIa, aadA2, bla*$_{NDM-5}$, *ble*$_{MBL}$, *dfrA12, mph(A), sul1, tetA, tmrB* | CP029978 |

aminoglycoside resistance genes: *aac(3)-IIa, aadA1*, *aadA2*; ß-lactam resistance genes: *bla*$_{CMY-2}$, *bla*$_{NDM-5}$, *bla*$_{TEM-30}$; bleomycin resistance gene: *ble*$_{MBL}$; macrolide resistance genes: *erm(B), mph(A)*; phenicol resistance gene: *floR;* sulfonamide and trimethoprim resistance genes: *sul1, sul2, dfrA1, dfrA12;* tetracycline resistance gene: *tetA;* tunicamycin resistance gene: *tmrB*.

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